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Comments on Draft Toxicological Review of Formaldehyde—Inhalation
Dear United States Environmental Protection Agency:

I recently reviewed the US Environmental Protection Agency (USEPA) Draft document “Toxicological Review of Formaldehyde - Synthesis of Evidence for Effects on the Respiratory System” Subsection on “Evaluation of experimental support for the hypothesized mode of action” and I believe there is an issue with the interpretation of a manuscript that I was the senior author.

- Leslie Recio, Susan Sisk, Linda Pluta, Edilberto Bermudez, Elizabeth A. Gross, Zhuchu Chen, Kevin Morgan, Cheryl Walker (1992) p53 Mutations in Formaldehyde-induced Nasal Squamous Cell Carcinomas in Rats. *Cancer Res* 52 (21): 6113–6116.

In this study, the p53 tumor suppressor gene (now referred to as TP53) squamous cell carcinomas (SCC) from formaldehyde exposed rats were sequenced using cDNA derived from mRNA extracted from each tumor. This study demonstrated that 5 of 11 of the rat nasal tumors analyzed had a point mutation in the coding region of p53. We concluded that since p53 mutation occurs in the development of many human SCCs, these data indicate that certain human and rat SCCs share a common step in development. A key observation of this study was that the point mutations observed in the cDNA were all homozygous point mutations exclusively at GC base pairs.

In normal rat tissues, both alleles of the p53 gene are expressed; however in the SCC from formaldehyde exposed rats there was a reduction to homozygosity at the p53 locus for the point mutation observed. To account for the homozygous point mutations observed, key observation indicates that the other allele of p53 in these SCC was either (1) not expressed or silenced; (2) or it has been deleted; (3) or that there has been a gene/conversion recombinational event rendering both alleles of the p53 gene homozygous; (4) or in these SCC p53 occurs as hemizygous single allele assuming a deletion or sister chromosome loss. Secondly, of the 5 rat nasal tumors with a point mutation in the coding region of p53, 2 were transitions G:C→A:T at CpG sites. CpG sites are the most common occurring single nucleotide polymorphism in the human genome, commonly occurring in broad spectrum human genetic disease and as part of the genome landscape of human cancers including SCC.

Of particular concern to me is the EPA interpretation of these data, from end stage tumors in 2yr old rats, as an indicator of a genotoxic mode-of-action. This interpretation is not plausible for several reasons.

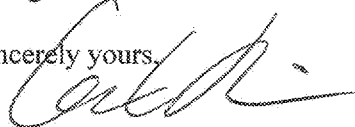
- DNA-protein crosslinks (DPC) formation occurs commonly in cells as a result of endogenous causes, such as reactions with aldehydes produced during cell metabolism, or exogenous sources and are composed of DNA, protein, and their cross-linked bonds. This is not an adduct that produces point mutations exclusively at GC base pairs in experimental systems.

- There is no mechanistic data to support the conclusion that the primary DNA lesion induced by formaldehyde in DNA – DNA-protein crosslinks – results in point mutations.
- Although genotoxicity is observed in certain respiratory epithelium in formaldehyde-exposed individual or rodents, these studies examined “large” forms of genetic damage, i.e., chromosomal aberrations or micronuclei visible by microscopy. Such chromosome-level changes cannot be mechanistically linked to the point mutations observed in SCC from formaldehyde exposed rats.
- In your review, you state “The absence of p53 mutations in reactive nasal mucosa after 90 days of exposure is consistent with p53 mutations acting as a selective or permissive factor acquired during the latter stages of formaldehyde-initiated carcinogenesis, facilitating increased genetic instability and the progression of nascent neoplasms to SCCs, which emerge months later (Hanahan and Weinberg, 2011, 2000).” However, p53 mutations are observed only in end stage tumors therefore do not meet the temporal expectation of US EPA’s criteria for a mutagenic carcinogen. The p53 mutations observed in Recio et al., 1992 meet criteria as cancer “passenger mutations” – part of the known genome instability producing thousands of mutations in cancers. There are no data to support that these late stage p53 mutations are genetic drivers of the SCC observed in formaldehyde exposed rats.
- The National Toxicology Program (NTP) tested formaldehyde in p53+/- heterozygous mice known to be responsive to genotoxic carcinogens (Morgan et al., 2017). Although you discuss this study, you misinterpret the finding by stating it supports “...a temporal relationship showing p53 mutations acting as a selective or permissive factor acquired during the latter stages of formaldehyde-initiated carcinogenesis, facilitating increased genetic instability and the progression of nascent neoplasms to SCCs, which emerge months later (Hanahan and Weinberg, 2011, 2000).” What the Morgan et al. (2017) reported was a squamous metaplasia of the respiratory epithelium of the nose at maximum tolerated doses of formaldehyde, a bioindicator of exposure. Formaldehyde did not cause nasal tumors or an increased prevalence of leukemia or lymphohematopoietic cancer in this study. In fact, NTP concluded that “The results of this short-term carcinogenicity study do not support a role for Trp53 in formaldehyde-induced neoplasia.

To conclude, the p53 point mutations observed in the Recio et al. (1992) study, which EPA cites as evidence of a formaldehyde-induced initiating event in their draft document, is not plausible for several reasons outlined above. There is no pathway for DPC to cause the point mutations observed exclusively at GC base pairs. Genotoxicity observed in respiratory tissues are not measures of mutagenic events, particularly point mutations: genotoxicity ≠ mutagenicity. There is no evidence to support a temporal nature of a mutagenic carcinogen as outlined by EPA. Finally, as determined by NTP, there is no data to support a role for p53 in formaldehyde-induced neoplasia.

Although I recognize that p53 mutations are considered hallmarks of cancer, the concern I have is the misrepresentation in your review of the Recio et al., 1992 manuscript indicating that these late occurring mutations are caused by formaldehyde induced DNA lesions. With the lack of any data to support a mutagenic event prior to tumor outcome in the target tissues, I conclude that the p53 mutations observed in SCC from formaldehyde exposed rats are late occurring passenger mutations resulting from known genome instability that occurs in cancers, are not formaldehyde-induced point mutations and do not support a mutagenic mode-of-action for tumor outcomes from formaldehyde exposed rats.

Sincerely yours,



Leslie Recio PhD DABT